Carbohydrate Polymers 137 (2016) 600–607

Contents lists available at ScienceDirect

Carbohydrate Polymers

journal homepage: www.elsevier.com/locate/carbpol

Synthesis of carboxymethyl chitin in aqueous solution and its thermo- and pH-sensitive behaviors

Hui Liu, Qizhi Yang, Lina Zhang, Renxi Zhuo, Xulin Jiang*

Key Laboratory of Biomedical Polymers of Ministry of Education & Department of Chemistry, Wuhan University, Luojia Hill, Wuhan 430072, PR China

ARTICLE INFO

Article history:
Received 10 September 2015
Received in revised form 3 November 2015
Accepted 6 November 2015
Available online 10 November 2015

Keywords:
Homogeneous synthesis
Proton NMR
Carboxymethyl chitin
Thermo-responsiveness
pH-responsiveness
Injectable hydrogel

ABSTRACT

Homogenous modification of natural chitin offers the advantage of fair structure control. In this work, novel carboxymethyl chitins (CMCHs) with broad range of degree of substitution (DS: 0.035 to 0.74), high degree of acetylation (DA) and little de-polymerization were synthesized homogeneously in aqueous NaOH/urea solution. The simultaneous determination of DA, DS and carboxymethylation fraction at C3 and C6 for these CMCHs was achieved by proton NMR in acidic deuterated aqueous solution for the first time. Due to the good homogeneity, the prepared CMCH-4 with lower DS of carboxymethylation exhibited, for the first time to our knowledge, dual thermo- and pH-sensitive properties. The nontoxic thermo-sensitive polymer systems gel at body temperature (37 °C) in physiological condition, which is very useful as injectable hydrogels for drug delivery and tissue engineering.

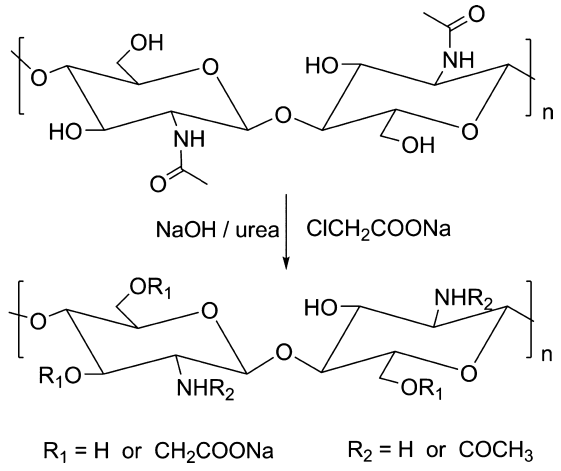
1. Introduction

Soluble carboxymethyl chitin (CMCH) is one of the most attractive derivatives of chitin for biomedical applications because the very poor solubility of chitin in all common solvents (Pillai, Paul, & Sharma, 2009) and limited solubility of chitosan in water and other organic solvents (Upadhyaya, Singh, Agarwal, & Tewari, 2014). It has been reported that CMCH can decrease the adsorption of blood components (Tokura, Hasegawa, Nishimura, Nishi, & Takatori, 1987), promote chondrogenesis (Kariya et al., 2007, 2010), induce bone formation (Sagar, Soni, & Bellare, 2012; Taniyama et al., 2013; Tokura & Tamura, 2001) and possesses sustained anti-microbial capability (Loke, Lau, Yong, Khor, & Sum, 2000). Moreover, CMCH can be degraded *in vivo* without formation of toxic degradation products (Tomihata & Ikada, 1997). To date, carboxymethyl chitin has been investigated for anti-obesity (Kong, Kim, Bak, Byun, & Kim, 2011), drug delivery (Dev et al., 2010; Huang, Wu, Wei, Chen, & Liu, 2012; Upadhyaya et al., 2014), wound dressing (Loke et al., 2000; Zhao, Wu, Chen, & Xing, 2015), and tissue engineering (Sagar et al., 2012; Taniyama et al., 2013). However, new method on synthesis and characterization of CMCH has been rather limited (Domard, 2011; Kumirska et al., 2010; Pillai et al., 2009), probably due to the characteristics crystalline structures with the strong inter- and intra-molecular hydrogen bonding between chitin chains. Traditional synthesis method of carboxymethyl chitin involved chitin slurry in the presence of concentrated NaOH (40–60% w/w) and isopropanol under the heterogeneous reaction conditions at elevated temperatures (Huang, Wu, Wei, Liao, & Chen, 2010; Sini, Santhosh, & Mathew, 2005). There are several disadvantages in the heterogeneous carboxymethylation of chitin: polymer degradation due to the harsh reaction conditions, increasing the degree of deacetylation resulted from the concentrated NaOH and difficulty in regioselective substitution, and poor control of compositional distribution of CMCH. Therefore, heterogeneous reactions typically resulted in limited functionalized CMCH that limits its application.

In order to enable carboxymethylation of chitin to proceed in a controlled manner, it is desirable to carry out the reaction in homogenous solution under mild conditions. Recently, alkali (NaOH or LiOH)/urea aqueous systems have been studied as a "green" solvent for the dissolution of chitin, from which a series of biocompatible chitin-based materials have been directly constructed (Chang, Chen, & Zhang, 2011; Chang et al., 2013; Duan et al., 2015; Fang et al., 2015), However, the derivatization of chitin in this "green" solvent system was rarely reported (Ding et al., 2012, 2013, 2015). Moreovere, the precise determination of the degree of acetylation (DA), degree of substitution of carboxymethylation (DS) and its distribution fraction of CMCH is very challengeable, but very critical. Several techniques have been developed to determine them, including IR and NMR spectroscopy (Kasaai, 2009), titration (Chen, Du, Wu, & Xiao, 2002; Ding et al., 2015; Huang et al., 2010; Kong, 2012), elemental analysis (Huang et al., 2010; Tokura, Nishi, Tsutsumi, & Somorin, 1983), and differential scanning calorimetry (DSC) (Kittur, Prashanth, Sankar, & Tharanathan,

* Corresponding author. Tel.: +00 86 27 68755200; fax: +00 86 27 68754509.
E-mail address: xljiang@whu.edu.cn (X. Jiang).

http://dx.doi.org/10.1016/j.carbpol.2015.11.025
0144-8617/



Scheme 1. Synthesis route of carboxymethyl chitin in NaOH/urea aqueous solution.

2002). Until now, there is no unique technique that can measure the DA with a high precision in the entire range (Kasaai, 2009). Also no reported method can provide full and accurate information for DA, DS and distribution fraction of carboxymethyl groups at C3 and C6 for CMCH so far (Kasaai, 2010; Kong, 2012). Therefore, exploring a method which can be applied for both water-soluble and water-insoluble CMCH with a high precision is very desirable.

In this work, to construct biocompatible smart hydrogel for biomedical applications, a series of new finely structurally controlled CMCHs with environment-responsiveness (thermo- and/or pH-sensitive properties) were synthesized homogeneously in the "green" solvent (NaOH/urea aqueous solution) based on the bioactive natural chitin as shown in Scheme 1. A novel method to characterize the detail structure information (DA, DS and distribution fraction of carboxymethyl groups) of these CMCHs facilely and precisely by ^{1}H NMR was reported. The dual thermo- and pH-sensitive properties and cytotoxicity of the resulted CMCHs were investigated and discussed.

2. Material and methods

2.1. Materials

Chitin powder was purchased from Golden-Shell Biochemical Co., Ltd. (Zhejiang, China). The viscosity-average molecular weight (M_η) was determined to be 7.56×10^5 Da according to the Mark–Houwink equation reported (Li et al., 2010). Deuterium chloride (DCl, 99.5% D; 20% w/w) was purchased from CIL (Cambridge Isotope Laboratories, MA, USA). DCl (99% D; acidity 37.5%) was purchased from Beijing Boya Dabei Technology Development (China). TSP (2,2,3,3-d4-3-(trimethylsilyl) propionic acid sodium salt, 98% D) was purchased from Alfa Aesar (MA, USA). All other chemicals were reagent grade and used without further purification.

2.2. Homogeneous synthesis of carboxymethyl chitin

Carboxymethyl chitin (CMCH) was homogeneously synthesized by one-pot reaction. Chitin was dissolved in 11 wt% NaOH/4 wt% urea aqueous solution according to a reported procedure (Chang et al., 2011). Different degrees of substitution (DS) of CMCH samples were obtained, named as CMCH-1–CMCH-9 (Table 1). Taking CMCH-1 as example, 2.3 g sodium monochloroacetate was added to 4 wt% chitin solution (100 g) at 2 °C under mechanical stirring. The reaction system was maintained at 15 °C for 24 h. The reaction system was transparent during the whole reaction proceeding. The reaction mixture was neutralized with HCl in an ice-bath, dialyzed against distilled water for one week and freeze dried.

2.3. Heterogeneous synthesis of CMCH

As comparison, CMCH was also prepared through the traditional heterogeneous reaction as reported with slight modification (Sini et al., 2005). A unit of 5 g chitin was mixed well with 40 mL 40% (w/w) NaOH and kept overnight at −20 °C. 200 mL isopropanol was added into the thawed chitin slurry. 28.8 g sodium monochloroacetate was added under stirring and the reaction system was stirred at 35 °C for 6 h and neutralized by HCl. The mixture was filtered, and the solid was collected, washed using 80% (v/v) alcohol/water and vacuum dried.

2.4. General characterization

Water solubility of the CMCHs was evaluated with polymer concentration of 10 mg/mL in distilled water overnight at room temperature.

Fourier transform infrared spectra (FTIR) were recorded over a wavenumber range 400 to 4000 cm^{-1} at room temperature on a FTIR spectrometer (Perkin-Elmer Spectrum One FTIR spectrometer). CMCHs were acidized as reported: 0.1 g CMCH was suspended in 20 mL 80% (v/v) ethyl alcohol aqueous solution, and 1 mL HCl (37% w/w) was added. After stirring for 30 min, the acidified CMCH was filtered, rinsed in 70–90% (v/v) ethyl alcohol to neutral, and vacuum dried (Chen & Park, 2003).

The wide-angle X-ray diffraction (XRD) measurements were carried out on a XRD diffractometer (D8-Advance, Bruker, USA). The patterns with Cu-$K\alpha$ radiation ($\lambda = 0.154$ nm) at 40 kV and 30 mA were recorded in the region of 2θ from 4° to 40° at a scanning rate of 2° min^{-1}. The samples were cut into powder and vacuum dried at 60 °C for 48 h before test.

Proton nuclear magnetic resonance (^{1}H NMR) spectra were determined with Mercury VX-300 spectrometer (300 MHz, Varian, USA) at 25 °C. TSP was used as an internal standard (at 0.00 ppm). CMCHs were dissolved in 20% DCl at 4 °C overnight at the polymer concentration of 20 mg/mL or hydrolyzed in 20% (w/w) DCl at 50 °C for 36 h before test. Chitin was hydrolyzed in concentrated DCl (37.5% w/w) at 50 °C for 36 h before test. 2-Methoxyacetic acid was dissolved in various concentration DCl at room temperature at the concentration of 20 mg/mL.

The molecular weight (M_w) of CMCH-9 was determined by size exclusion chromatography combined with multi-angle light scattering (SEC-MALS) system using a Waters 2690D separation module, a Waters 2414 refractive index (RI) detector and a Wyatt DAWN EOS MALS detector. Two chromatographic columns (Waters Ultrahydrogel™ 2000, 7.8 mm × 300 mm) and PBS (0.01 M, pH = 7.4, 150 mM ionic strength) as eluent at a flow rate of 0.5 mL/min were used at 25 °C. The data were processed using Astra software (Wyatt Technology).

2.5. Temperature/pH-responsive behavior of the polymer

Optical transmittance for CMCH solution at different pH was measured by UV (Perkin-Elmer Lambda Bio 40 UV–Vis spectrometer) at 650 nm to evaluate the pH-responsive behavior of CMCHs. The sample was firstly dissolved in 1.0 M NaOH at 10 mg/mL, and the solution pH was adjusted with 6.0 M HCl in ice bath.

Dynamic light scattering (DLS) was used to measure the thermo-responsive behavior of the polymer by a Zetasizer Nano series Nano ZS ZEN3600 with a 633 nm laser and 173° backscatter detection optics (Malvern Instrument). The CMCH solutions were prepared at concentrations of 4 mg/mL in 1.0 M NaOH and solution pH was adjusted with 6.0 M HCl in ice bath. The polymer solution was filtered through a 0.45 μm Millipore filter before measurement. The light-scattering intensity was recorded continuously by heating the